

Figure 1A

[illegible]

Figure 1B

401	M4cDNA	TGATTGGGAA	AAAAACCGAC	AAGCATTAGC	CAAAATGCGC	CAAGTTTTTG	CAAAGGGAAC	AACTGGCGGA	TTGGGAGGGG	480
	M6cDNA	TGATTGGGAA	AAAAACCGAC	AAGCATTAGC	CAAAATGCGC	CAAGTTTTTG	CAAAGGGAAC	AACTGGCGGA	TTGGGAGGGG	
	M8cDNA	TGATTGGGAA	AAAAACCGAC	AAGCATTAGC	CAAAATGCGC	CAAGTTTTTG	CAAAGGGAAC	AACTGGCGGA	TTGGGAGGGG	
	M15cDNA	TGATTGGGAA	AAAAACCGAC	AAGCATTAGC	CAAAATGCGC	CAAGTTTTTG	CAAAGGGAAC	AACTGGCGGA	TTGGGAGGGG	
	Consensus	TGATTGGGAA	AAAAACCGAC	AAGCATTAGC	CAAAATGCGC	CAAGTTTTTG	CAAAGGGAAC	AACTGGCGGA	TTGGGAGGGG	
481	M4cDNA	AAATCTACGT	GGTGACTGAT	TGTTTCAGATG	ACAAATGCTGC	AAATCCAAAG	CCAGGGACAC	TTCGTTGTGG	TGTCACCCAA	560
	M6cDNA	AAATCTACGT	GGTGACTGAT	TGTTTCAGATG	ACAAATGCTGC	AAATCCAAAG	CCAGGGACAC	TTCGTTGTGG	TGTCACCCAA	
	M8cDNA	AAATCTACGT	GGTGACTGAT	TGTTTCAGATG	ACAAATGCTGC	AAATCCAAAG	CCAGGGACAC	TTCGTTGTGG	TGTCACCCAA	
	M15cDNA	AAATCTACGT	GGTGACTGAT	TGTTTCAGATG	ACAAATGCTGC	AAATCCAAAG	CCAGGGACAC	TTCGTTGTGG	TGTCACCCAA	
	Consensus	AAATCTACGT	GGTGACTGAT	TGTTTCAGATG	ACAAATGCTGC	AAATCCAAAG	CCAGGGACAC	TTCGTTGTGG	TGTCACCCAA	
561	M4cDNA	GATAAACCTT	TGTGGATCAT	CTTTAAGAAA	GATATGGTCA	TAAACCTTAA	ACACGAGCTT	GTGATAAACA	AAGACAAGAC	640
	M6cDNA	GATAAACCTT	TGTGGATCAT	CTTTAAGAAA	GATATGGTCA	TAAACCTTAA	ACACGAGCTT	GTGATAAACA	AAGACAAGAC	
	M8cDNA	GATAAACCTT	TGTGGATCAT	CTTTAAGAAA	GATATGGTCA	TAAACCTTAA	ACACGAGCTT	GTGATAAACA	AAGACAAGAC	
	M15cDNA	GATAAACCTT	TGTGGATCAT	CTTTAAGAAA	GATATGGTCA	TAAACCTTAA	ACACGAGCTT	GTGATAAACA	AAGACAAGAC	
	Consensus	GATAAACCTT	TGTGGATCAT	CTTTAAGAAA	GATATGGTCA	TAAACCTTAA	ACACGAGCTT	GTGATAAACA	AAGACAAGAC	
641	M4cDNA	AATTGATGGA	AGAGGTGCAA	ATGTTGAGAT	CACCTTGTTGGC	GGTCTCACCA	TTCACAACGT	TTGCAATGTG	ATCATTTCATA	720
	M6cDNA	AATTGATGGA	AGAGGTGCAA	ATGTTGAGAT	CACCTTGTTGGC	GGTCTCACCA	TTCACAACGT	TTGCAATGTG	ATCATTTCATA	
	M8cDNA	AATTGATGGA	AGAGGTGCAA	ATGTTGAGAT	CACCTTGTTGGC	GGTCTCACCA	TTCACAACGT	TTGCAATGTG	ATCATTTCATA	
	M15cDNA	AATTGATGGA	AGAGGTGCAA	ATGTTGAGAT	CACCTTGTTGGC	GGTCTCACCA	TTCACAACGT	TTGCAATGTG	ATCATTTCATA	
	Consensus	AATTGATGGA	AGAGGTGCAA	ATGTTGAGAT	CACCTTGTTGGC	GGTCTCACCA	TTCACAACGT	TTGCAATGTG	ATCATTTCATA	
721	M4cDNA	ACATTTCACAT	ACATGATATT	AAAGTAACCG	AAGGTGGAAT	TATTAAGGCA	ACGGACGCTA	AACCAGGACA	TAGACATAAG	800
	M6cDNA	ACATTTCACAT	ACATGATATT	AAAGTAACCG	AAGGTGGAAT	TATTAAGGCA	ACGGACGCTA	AACCAGGACA	TAGACATAAG	
	M8cDNA	ACATTTCACAT	ACATGATATT	AAAGTAACCG	AAGGTGGAAT	TATTAAGGCA	ACGGACGCTA	AACCAGGACA	TAGACATAAG	
	M15cDNA	ACATTTCACAT	ACATGATATT	AAAGTAACCG	AAGGTGGAAT	TATTAAGGCA	ACGGACGCTA	AACCAGGACA	TAGACATAAG	
	Consensus	ACATTTCACAT	ACATGATATT	AAAGTAACCG	AAGGTGGAAT	TATTAAGGCA	ACGGACGCTA	AACCAGGACA	TAGACATAAG	

Figure 1C

801	M4cDNA	AGCGACGGAG	ATGGTATTGG	TGTTGCTGGT	TCTTCAAAGA	TATGGATCGA	TCATTGCACA	CTTAGTCAATG	GTCCAGATGG	880
	M6cDNA	AGCGACGGAG	ATGGTATTGG	TGTTGCTGGT	TCTTCAAAGA	TATGGATCGA	TCATTGCACA	CTTAGTCAATG	GTCCAGATGG	
	M8cDNA	AGCGACGGAG	ATGGTATTGG	TGTTGCTGGT	TCTTCAAAGA	TATGGATCGA	TCATTGCACA	CTTAGTCAATG	GTCCAGATGG	
	M15cDNA	AGCGACGGAG	ATGGTATTGG	TGTTGCTGGT	TCTTCAAAGA	TATGGATCGA	TCATTGCACA	CTTAGTCAATG	GTCCAGATGG	
	Consensus	AGCGACGGAG	ATGGTATTGG	TGTTGCTGGT	TCTTCAAAGA	TATGGATCGA	TCATTGCACA	CTTAGTCAATG	GTCCAGATGG	
881	M4cDNA	CCTTATTGAT	GTCACGTTGG	GTAGCACAGC	CGTTACCAAT	TCCAATTGCA	AATTTAGCCA	TCACCAAAAA	ATTCTATTAC	960
	M6cDNA	CCTTATTGAT	GTCACGTTGG	GTAGCACAGC	CGTTACCAAT	TCCAATTGCA	AATTTAGCCA	TCACCAAAAA	ATTCTATTAC	
	M8cDNA	CCTTATTGAT	GTCACGTTGG	GTAGCACAGC	CGTTACCAAT	TCCAATTGCA	AATTTAGCCA	TCACCAAAAA	ATTCTATTAC	
	M15cDNA	CCTTATTGAT	GTCACGTTGG	GTAGCACAGC	CGTTACCAAT	TCCAATTGCA	AATTTAGCCA	TCACCAAAAA	ATTCTATTAC	
	Consensus	CCTTATTGAT	GTCACGTTGG	GTAGCACAGC	CGTTACCAAT	TCCAATTGCA	AATTTAGCCA	TCACCAAAAA	ATTCTATTAC	
961	M4cDNA	TCGGAGCAGA	CAATTACAT	GTAGACGATA	AAAAAATGCA	TGTCACAGTA	GCATTCAACA	GGTTCGCAGA	AGCATGTGAT	1040
	M6cDNA	TCGGAGCAGA	CAATTACAT	GTAGACGATA	AAAAAATGCA	TGTCACAGTA	GCATTCAACA	GGTTCGCAGA	AGCATGTGAT	
	M8cDNA	TCGGAGCAGA	CAATTACAT	GTAGACGATA	AAAAAATGCA	TGTCACAGTA	GCATTCAACA	GGTTCGCAGA	AGCATGTGAT	
	M15cDNA	TCGGAGCAGA	CAATTACAT	GTAGACGATA	AAAAAATGCA	TGTCACAGTA	GCATTCAACA	GGTTCGCAGA	AGCATGTGAT	
	Consensus	TCGGAGCAGA	CAATTACAT	GTAGACGATA	AAAAAATGCA	TGTCACAGTA	GCATTCAACA	GGTTCGCAGA	AGCATGTGAT	
1041	M4cDNA	CAAGAATGC	CACGATGTCG	ATTGGATT	TTCCAAGTTG	TTAACAAATGA	CTACACCAGC	TGGGGAACGT	ACGCCATTGG	1120
	M6cDNA	CAAGAATGC	CACGATGTCG	ATTGGATT	TTCCAAGTTG	TTAACAAATGA	CTACACCAGC	TGGGGAACGT	ACGCCATTGG	
	M8cDNA	CAAGAATGC	CACGATGTCG	ATTGGATT	TTCCAAGTTG	TTAACAAATGA	CTACACCAGC	TGGGGAACGT	ACGCCATTGG	
	M15cDNA	CAAGAATGC	CACGATGTCG	ATTGGATT	TTCCAAGTTG	TTAACAAATGA	CTACACCAGC	TGGGGAACGT	ACGCCATTGG	
	Consensus	CAAGAATGC	CACGATGTCG	ATTGGATT	TTCCAAGTTG	TTAACAAATGA	CTACACCAGC	TGGGGAACGT	ACGCCATTGG	
1121	M4cDNA	TGGTAGTGCC	AATCCTACTA	TCCTTAGCCA	AGGCAACCGA	TTCCATGCTC	CGAATGACCC	AATGAAGAAA	AATGTGTTGG	1200
	M6cDNA	TGGTAGTGCC	AATCCTACTA	TCCTTAGCCA	AGGCAACCGA	TTCCATGCTC	CGAATGACCC	AATGAAGAAA	AATGTGTTGG	
	M8cDNA	TGGTAGTGCC	AATCCTACTA	TCCTTAGCCA	AGGCAACCGA	TTCCATGCTC	CGAATGACCC	AATGAAGAAA	AATGTGTTGG	
	M15cDNA	TGGTAGTGCC	AATCCTACTA	TCCTTAGCCA	AGGCAACCGA	TTCCATGCTC	CGAATGACCC	AATGAAGAAA	AATGTGTTGG	
	Consensus	TGGTAGTGCC	AATCCTACTA	TCCTTAGCCA	AGGCAACCGA	TTCCATGCTC	CGAATGACCC	AATGAAGAAA	AATGTGTTGG	



Figure 1D

M4cDNA	1201	TGAGGGCTGA	TGCACCAT	ACAGAGTCAA	TGAAGTGGAA	TTGGAGATCT	GAGAAAGACT	TGTTAGAAAA	1280	TGGAGCTATA
M6cDNA		TGAGGGCTGA	TGCACCAT	ACAGAGTCAA	TGAAGTGGAA	TTGGAGATCT	GAGAAAGACT	TGTTAGAAAA		TGGAGCTATA
M8cDNA		TGAGGGCTGA	TGCACCAT	ACAGAGTCAA	TGAAGTGGAA	TTGGAGATCT	GAGAAAGACT	TGTTAGAAAA		TGGAGCTATA
M15cDNA		TGAGGGCTGA	TGCACCAT	ACAGAGTCAA	TGAAGTGGAA	TTGGAGATCT	GAGAAAGACT	TGTTAGAAAA		TGGAGCTATA
Consensus		TGAGGGCTGA	TGCACCAT	ACAGAGTCAA	TGAAGTGGAA	TTGGAGATCT	GAGAAAGACT	TGTTAGAAAA		TGGAGCTATA
M4cDNA	1281	TTTGTAGCAT	CAGGGTGCGA	CCCGCATCTA	ACCCCGGAAC	AAAAAAGCCA	TTTGATTCCA	GCTGAACCAG	1360	GATCAGCAGT
M6cDNA		TTTGTAGCAT	CAGGGTGCGA	CCCGCATCTA	ACCCCGGAAC	AAAAAAGCCA	TTTGATTCCA	GCTGAACCAG		GATCAGCAGT
M8cDNA		TTTGTAGCAT	CAGGGTGCGA	CCCGCATCTA	ACCCCGGAAC	AAAAAAGCCA	TTTGATTCCA	GCTGAACCAG		GATCAGCAGT
M15cDNA		TTTGTAGCAT	CAGGGTGCGA	CCCGCATCTA	ACCCCGGAAC	AAAAAAGCCA	TTTGATTCCA	GCTGAACCAG		GATCAGCAGT
Consensus		TTTGTAGCAT	CAGGGTGCGA	CCCGCATCTA	ACCCCGGAAC	AAAAAAGCCA	TTTGATTCCA	GCTGAACCAG		GATCAGCAGT
M4cDNA	1361	TCTTCAACTC	ACCAGTTGTG	CTGGCACGCT	CAAAATGCGTT	CCTGGAAAAC	CTTGTTAATA	GTTATCACCC	1440	ACTTTTITTT
M6cDNA		TCTTCAACTC	ACCAGTTGTG	CTGGCACGCT	CAAAATGCGTT	CCTGGAAAAC	CTTGTTAATA	GTTATCACCC		ACTTTTITTT
M8cDNA		TCTTCAACTC	ACCAGTTGTG	CTGGCACGCT	CAAAATGCGTT	CCTGGAAAAC	CTTGTTAATA	GTTATCACCC		ACTTTTITTT
M15cDNA		TCTTCAACTC	ACCAGTTGTG	CTGGCACGCT	CAAAATGCGTT	CCTGGAAAAC	CTTGTTAATA	GTTATCACCC		ACTTTTITTT
Consensus		TCTTCAACTC	ACCAGTTGTG	CTGGCACGCT	CAAAATGCGTT	CCTGGAAAAC	CTTGTTAATA	GTTATCACCC		ACTTTTITTT
M4cDNA	1441	TTTATTGTTA	TTAGCATTTT	TTTACTTTGT	TAGGATTGTA	GTGGAATGAG	ACATTGATAC	GTGCATTACA	1520	AGACCATTCa
M6cDNA		TTTATTGTTA	TTAGCATTTT	TTTACTTTGT	TAGGATTGTA	GTGGAATGAG	ACATTGATAC	GTGCATTACA		AGACCATTCa
M8cDNA		TTTATTGTTA	TTAGCATTTT	TTTACTTTGT	TAGGATTGTA	GTGGAATGAG	ACATTGATAC	GTGCATTACA		AGACCAAAAA
M15cDNA		TTTATTGTTA	TTAGCATTTT	TTTACTTTGT	TAGGATTGTA	GTGGAATGAG	ACATTGATAC	GTGCATTACA		AGACCAAAAA
Consensus		TTTATTGTTA	TTAGCATTTT	TTTACTTTGT	TAGGATTGTA	GTGGAATGAG	ACATTGATAC	GTGCATTACA		AGACCATtc
M4cDNA	1521	TCAACATATT	TTGCTACTAT	CACATGTTCA	CGTTAAAAAA	AAAAA	1570	AAAAA		AAAAA
M6cDNA		TCAACATATT	TTGCTAAAA-	-----	-----	-----	-----	-----		-----
M8cDNA		AAAAA	AAAAA	AAAAA	AAAAA	AAAAA	AAAAA	AAAAA		AAAAA
M15cDNA		AAAAA	AAAAA	AAAAA	AAAAA	AAAAA	AAAAA	AAAAA		AAAAA
Consensus		tCAACAtAtt	ttgcta..a.

Figure 2

cDNA and amino acid sequences of M4, M6, M15 (identical clones):

```
atggaaaaacattatTTTgttatattgttcaccgcagcgtttgttttcgtgggtgcagct
M E K H Y F V I L F T A A F V F V G A A
gctcgggctgacattggtgatgagctcgaagcggctcaatttaattcaacaaggaggggc
A R A D I G D E L E A A Q F N S T R R G
ttacacgaatgtgcagcacataacataatagacaagtgttgagggtgcaaagctgattgg
L H E C A A H N I I D K C W R C K A D W
gaaaaaaaccgacaagcattagccaaatgcgcgcaagggttttgcaaagggaacaactggc
E K N R Q A L A K C A Q G F A K G T T G
ggattgggaggggaaatctacgtggtgactgattgttcagatgacaatgctgcaaatcca
G L G G E I Y V V T D C S D D N A A N P
aagccagggacacttcgttgtggtgtcaccgaagataaacctttgtggatcatctttaag
K P G T L R C G V T Q D K P L W I I F K
aaagatatggtcataaaacttaaacacgagcttgtgataaacaagacaagacaattgat
K D M V I K L K H E L V I N K D K T I D
ggaagaggtgcaaattgttgagatcacttgtggcgggtctcaccattcacaacgtttgcaat
G R G A N V E I T C G G L T I H N V C N
gtgatcattcataacattcacatacatgatattaaagtaaccgaaggtggaattattaag
V I I H N I H I H D I K V T E G G I I K
gcaacggacgctaaaccaggacatagacataagagcgacggagatggtatttgtgttgct
A T D A K P G H R H K S D G D G I C V A
ggttcttcaaagatatggatcgatcattgcacacttagtcatggtccagatggccttatt
G S S K I W I D H C T L S H G P D G L I
gatgtcacgttgggtagcacagccgttaccattttccaattgcaaatttagccatcaccaa
D V T L G S T A V T I S N C K F S H H Q
aaaattctattactcggagcagacaattcacatgtagacgataaaaaaatgcatgtcaca
K I L L L G A D N S H V D D K K M H V T
gtagcattcaacaggttcgcagaagcatgtgatcaaagaatgccacgatgtcgatttgga
V A F N R F A E A C D Q R M P R C R F G
tttttccaagttgttaacaatgactacaccagctggggaacgtacgccattggtggtagt
F F Q V V N N D Y T S W G T Y A I G G S
gccaatcctactatccttagccaaggcaaccgattccatgctccgaatgacccaatgaag
A N P T I L S Q G N R F H A P N D P M K
aaaaatgtgttggtgagggctgatgcaccacatacagagtcaatgaagtgggaattggaga
K N V L V R A D A P H T E S M K W N W R
tctgagaaagacttgttagaaaaatggagctatatTTTgtagcatcaggggtgcgacccgcat
S E K D L L E N G A I F V A S G C D P H
ctaaccccggaaacaaaaaagccatttgattccagctgaaccaggatcagcagttcttcaa
L T P E Q K S H L I P A E P G S A V L Q
ctcaccagttgtgctggcagcgtcaaatgcgttcctggaaaaccttgtaa
L T S C A G T L K C V P G K P C -
```

Figure 3**cDNA and amino acid sequence of clone M8:**

atggaaaaacattatTTTTgttatattgttcaccgcagcgtttgttttcgtgggtgcagct
M E K H Y F V I L F T A A F V F V G A A
gctcgggctgacattggtgatgagctcgaagcggctcaattttaattcaacaaggaggggc
A R A D I G D E L E A A Q F N S T R R G
ttacacgaatgtgcagcacataacataatagacaagtgttgagggtgcaaagctgattgg
L H E C A A H N I I D K C W R C K A D W
gaaaaaaaccgacaagcattagccaaatgcgcgcaagggttttgcaaagggaacaactggc
E K N R Q A L A K C A Q G F A K G T T G
ggattgggaggggaaatctacgtggtgactgattgttcagatgacaatgctgcaaattcca
G L G G E I Y V V T D C S D D N A A N P
aagccagggacacttcgttgtggtgtcacccaagataaacctttgtggatcatcttcaag
K P G T L R C G V T Q D K P L W I I F K
aaagatatggtcataaaacttaaacacgagcttgtgataaacaagacaagacaattgat
K D M V I K L K H E L V I N K D K T I D
ggaagaggtgcaaattgttgagatcacttgtggcgggtctcaccattcacaacgtttgcaat
G R G A N V E I T C G G L T I H N V C N
gtgatcattcataacattcacatacatgatattaaagtaacggaagggtggaattattaag
V I I H N I H I H D I K V T E G G I I K
gcaacggacgctaaaccagggcatagacataagagcgacggagatggtattttgtgttgct
A T D A K P G H R H K S D G D G I C V A
ggttcttcgaagatatggatcgatcattgcacacttagtcattggtccagatggccttatt
G S S K I W I D H C T L S H G P D G L I
gatgtcacgttgggtagcacagccgttaccattttccaattgcaaatttagccatcaccaa
D V T L G S T A V T I S N C K F S H H Q
aaaattctattactcggagcagacaattcacatgtagacgataaaaaaatgcatgtcaca
K I L L L G A D N S H V D D K K M H V T
gtcgcattcaacaggttcgcagaagcatgtgatcaaagaatgccacgatgtcgatttgga
V A F N R F A E A C D Q R M P R C R F G
tttttccaagttgttaacaatgactacaccagctggggaacgtacgccattggtggttagc
F F Q V V N N D Y T S W G T Y A I G G S
gccaatcctactatccttagccaaggcaaccgattccatgctcccaatgacccaatgaag
A N P T I L S Q G N R F H A P N D P M K
aaaaatgtgttggtgagggtgatgcaccacatacagagtcaatgaagtgggaattggaga
K N V L V R A D A P H T E S M K W N W R
tctgagaaagacttgttagaaaaatggagctatatattttagcatcaggggtgcgacccgcat
S E K D L L E N G A I F V A S G C D P H
ctaaccgggaacaaaaaagccatttaattccagctgaaccaggatcagcagttcttcaa
L T P E Q K S H L I P A E P G S A V L Q
ctcaccagttgtgctggcagcgtcaaattgcgttcctggaaaaccttgtaa
L T S C A G T L K C V P G K P C -

Figure 4

pHIS-Parallel2

T7 Promoter-> _____ Lac Operator _____ Xba I
 GAAATTAATACGACTCACTATAGGGAATTGTGAGCGGATAACAATTCCCCTCTAGAAATAATTTGTTTAACTTTAAGAA

Nde I
 GGAGATATACAT ATG TCG TAC TAC CAT CAC CAT CAC CAT CAC GAT TAC GAT ATC CCA ACG ACC
 Met Ser Tyr Tyr His His His His His His Asp Tyr Asp Ile Pro Thr Thr
 6xHis Spacer Region

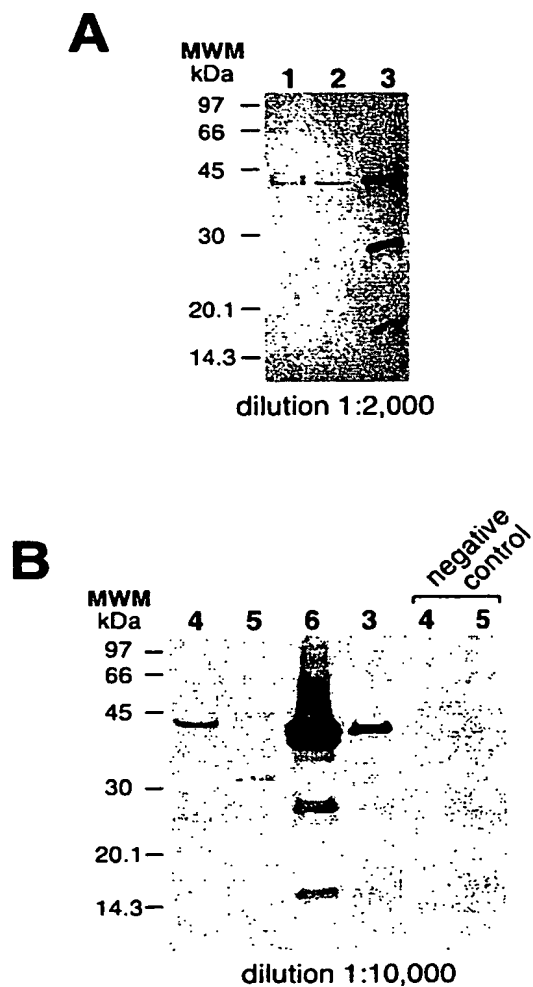
Ehe I Nco I Bam HI Eco RI Stu I Sal I Sst I
 GAA AAC CTG TAT TTT CAG GGC GCC ATG GGA TCC GGA ATT CAA AGG CCT ACG TCG ACG AGC
 Glu Asn Leu Tyr Phe Gln Gly Ala Met Gly Ser Gly Ile Gln Arg Pro Thr Ser Thr Ser
 rTEV Protease
 Cleavage Site —▲—

Spe I Not I Nsp V Xba I Pst I Xho I
 TCA ACT AGT GCG GCC GCT TTC GAA TCT AGA GCC TGC AGT CTC GAG CAC CAC CAC CAC CAC
 Ser Thr Ser Ala Ala Ala Phe Glu Ser Arg Ala Cys Ser Leu Glu His His His His His

CAC TGA GAT CCG GCT GCT AAC AAA GCC CGA AAG GAA GCT GAG TTG GCT GCT GCC ACC GCT
 His ***

*Non-unique sites

Figure 5

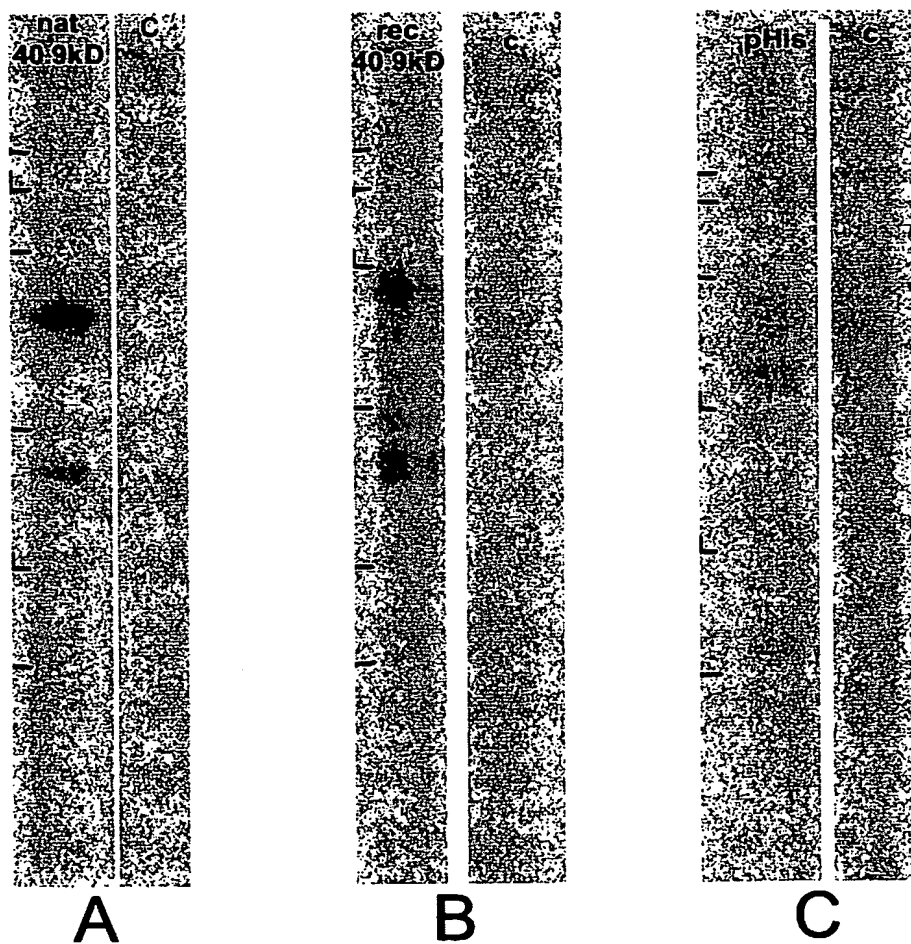
Immunoblot with rabbit anti-Amb a 1 antibodies

- 1 - Mugwort pollen extract
- 2 - Purified mugwort pollen allergen
- 3 - Purified Amb a 1 from ragweed pollen (natural Amb a 1)
- 4 - Recombinant mugwort allergen
- 5 - Control bacterial lysate
- 6 - Ragweed pollen extract

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Figure 6

IgE blot with NIH patient



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Figure 7



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